DOI: 10.4274/jcrpe.galenos.2020.2020.0093

research

Genotype and Phenotype Heterogeneity in Neonatal Diabetes: A Single Centre Experience in Turkey

Yasemin Denkboy Ongen[1], Erdal Eren[1], Ozgecan Demirbas[1], Elif Sobu[1], Sian Ellard[2], Omer Tarim[1], Elisa De Franco[3]
[1]Bursa Uludag University, School of Medicine, Department of Pediatric Endocrinology, Bursa, Turkey
[2]Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK
[3]Genomics Laboratory, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK

What is already known on this topic?
Neonatal diabetes mellitus is defined as diabetes diagnosed during the first 6 months of life. The most frequent mutations in Europe are reported to affect the ATP-dependent potassium channel genes. Genetic testing is essential for diagnosis and management.

What does this study add?
Neonatal diabetes mellitus can, in rare cases, arise after the age of six months. In our cohort, most of the patients had ATP-dependent potassium channel mutations similar to the literature. Genetic results in these patients result in improved treatment with a transition to sulphonylurea therapy.

Abstract
Introduction: Neonatal diabetes mellitus (NDM) may be transient or permanent, and the majority is caused by genetic mutations. Early diagnosis is essential to select the patients who will respond to oral treatment. In this investigation, we aimed to present the phenotype and genotype of our patients with NDM and share our experience in a single tertiary center.
Methods: A total of 16 NDM patients from 12 unrelated families are included in the study. The clinical presentation, age at the diagnosis, perinatal and family history, consanguinity, gender, Hemoglobin A1c (HbA1c), C-peptide, insulin, insulin autoantibodies, genetic mutations, and response to treatment are retrospectively evaluated.
Results: The median age at diagnosis of diabetes was five months (4 days-18 months), 6 patients with a confirmed genetic diagnosis were diagnosed after 6 months. Three patients had *KCNJ11* mutations, six had *ABCC8* mutations, three had *EIF2AK3* mutations, and one had a *de novo INS* mutation. All the permanent NDM patients with *KCNJ11* and *ABCC8* mutations were started on SU treatment resulting in a significant increase in C-peptide level, better glycemic control, and discontinuation of insulin.
Conclusions: Although NDM is defined as diabetes diagnosed during the first six months of life, and a diagnosis of type 1 diabetes is known much more common between the ages of 6 and 24 months, in rare cases it may present as late as 12 or even 24 months of age. Molecular diagnosis in NDM is important for planning treatment and predicting prognosis. Therefore, genetic testing is essential in these patients.

Yasemin Denkboy Ongen MD, Bursa Uludag University, School of Medicine, Department of Pediatric Endocrinology, Bursa, Turkey
+90 224 295 05 33
ydenkboyongen@uludag.edu.tr
ORCID ID: 0000-0002-5657-4260
15.05.2020
19.08.2020

Introduction
Diabetes presenting in the first six months of life is classified as neonatal diabetes mellitus (NDM) (1-4). Its incidence in Europe is reported to be 1:90,000 (5). NDM may be transient or permanent; about 50 to 60 percent of NDM is transient (2,3). Although most cases remit within three months after diagnosis, about 50% of the patients relapse later in life and most frequently during adolescence (5). Insulin treatment is usually required during the first few days at initial diagnosis, but it is life-long after relapse (3,4).
The incidence of permanent NDM in the Middle East is more than of Europe at 1:21,000 (6). Mutations in more than 25 genes have been reported to cause NDM so far (7,8). The most frequent mutations in Europe are reported to affect the pancreatic ATP-dependent potassium channel genes (*KCNJ11* and *ABCC8*), and most of them are spontaneous mutations (9). Early diagnosis is essential because NDM due to these mutations is responsive to sulphonylurea (SU) treatment, and early treatment improves neurocognitive development (10-15).
In this investigation, we present the genotypic and phenotypic characteristics of the patients with NDM, followed by the pediatric endocrinology clinic at Bursa Uludag University Hospital.

Subjects and Methods

Patients

A total of 16 NDM patients from 12 unrelated families, followed by the pediatric endocrinology clinic at Bursa Uludag University Hospital, are included in the study. Clinical data were obtained from medical records, and consent form was filled out by all parents and participants. Patients diagnosed with diabetes below the age of 12 months and/or had infantile diabetes with syndromic features and/or have a family history of NDM were included in the study. The clinical presentation, age at diagnosis, perinatal and family history, consanguinity, gender, glycated hemoglobin (HbA1c), C-peptide, insulin, and insulin autoantibodies, genetic mutations, and response to treatment are retrospectively evaluated. Informed consent for genetic testing was obtained from the parents. The study was approved by the Ethical Committee of Bursa Uludag University (approval number: 2020-8/23).

Laboratory analysis

Serum glucose was analyzed by spectrophotometric methods. C-peptide and insulin were assessed with chemiluminescent microparticle immunoassay (CMIA). HbA1c was measured by high-pressure liquid chromatography (HPLC). Glutamic acid decarboxylase antibody (GAD-65) and anti-insulin antibody were performed by enzyme immunoassay (EIA). Pancreatic islet cell antibody (ICA) was studied by indirect fluorescent antibody (IFA) method.

Genetic analysis

Analysis of all coding regions and exon/intron boundaries of the *KCNJ11*, *ABCC8*, *INS* and *EIF2AK3* genes has been performed by Sanger sequencing. Genetic testing for all known genetic causes of NDM for 8 patients was performed by the Exeter genomic laboratory, as previously described (16). The clinical significance of the variant was assessed using the ACGS Best Practice Guidelines for Variant Classification 2019 (17).

Statistical analyses

Statistical analysis was performed using IBM SPSS 21.0 for Windows statistical software.

Data were expressed as median (min-max range) or mean ± SD (range).

Results

The median age at diagnosis of diabetes was five months (4 days-18 months), and the F/M ratio was 1.3:1. The mean glucose level at diagnosis was 475±137 mg/dl—nine patients presented with diabetic ketoacidosis (DKA), two patients with ketosis, and 4 with hyperglycemia. One patient was diagnosed elsewhere, and the initial presentation was not known (patient number 12.15). The median HbA1c at the time of diagnosis was 10.2% (5.8-17.1%), and the median C-peptide was 0.085 ng/mL (0.01-1.22 ng/mL) (reference range 0.78-5.19 ng/ml). Eleven patients were born full-term, three of them with low birth weight (<2,500 g), and five with a birth weight of 2,500-3,500 g. The gestational age and birth weight of four patients were not available. Multiple insulin regimens such as intermediate-acting (NPH), rapid-acting (insulin lispro) and short-acting insulin (regular), were started most of the patients. Only one patient was treated with an insulin pump. Pancreatic imaging (sonographic examination) was performed all of NDM patients, and none of them showed pancreatic abnormality. A genetic test was performed in 15 patients (Table 1).

A mutation in a gene known to cause NDM was identified in thirteen patients, but for two patients testing for all the known NDM genes did not detect a likely causative mutation. These patients without a mutation identified were diagnosed at the age of seven months and four days, respectively and were both positive for anti-GAD antibodies (concentrations were 26.5 and 53.95 IU/mL, normal level <5 IU/mL) (patients number 7.9 and 9.11 in Table 1). Although anti-GAD antibodies positivity, anti-insulin antibodies were low (concentrations were 0.2 and 2.5 IU/mL, normal level 0-10 IU/mL). Their birth weights were 3,700 g and 2,300 g, and they were not significantly different from the rest of the cohort.

Three unrelated patients had the *KCNJ11* mutations, six (3 from the same family) had *ABCC8* mutations, three had *EIF2AK3* mutations, and one had a de novo *INS* mutation.

Patients with ATP-dependent potassium channel mutations

Patient 6.6 was diagnosed with ketosis at 18 months of age and was on insulin treatment until she was 17 years old when she was found to be homozygous for a previously reported *ABCC8* mutation classified as pathogenic (p.Glu382Lys) and switched to SU treatment (patient number 6.6). She had two cousins with diabetes on insulin treatment at 18 and 24 years of age who were also diagnosed during infancy (patients number 6.7 and 6.8). These patients were also found to be homozygous for the *ABCC8* pathogenic variant and switched to SU. These three patients all responded well to oral treatment, and insulin was successfully discontinued.

One patient diagnosed at twelve days of age with a previously reported *ABCC8* heterozygous mutation (p.Arg1183Gln) was off-treatment at four months of age, confirming transient NDM (patient number 8.10).

Two sisters diagnosed with NDM at 6 and 8 months of age were homozygote for the p.Trp231Leu mutation in the *ABCC8* gene (patients number 10.12 and 10.13). Although, this variant was not previously reported in the literature and initially classified as a variant of uncertain significance, a trial switch from insulin treatment to SU was successful and the variant could therefore be re-classified as likely pathogenic.

Three unrelated patients were found to be heterozygous for the previously reported pathogenic *KCNJ11* p.Val59Met mutation. This variant has been previously reported in patients with iDEND (18,19). However, none of our patients was reported to have neurological features at the ages of 7, 6.5 and 6.

All the permanent NDM patients with *KCNJ11* and *ABCC8* mutations were successfully transferred to SU treatment resulting in a significant increase in C-peptide level after 3 months later, better glycemic regulation, and discontinuation of insulin (Table 2). SU was started at a dose of 0.2 mg/kg/day, twice a day. Later, doses were adjusted with blood glucose levels. The doses of SU was in the range of 0.2-1.2 mg/kg/day. Only one patient required a single dose of long-acting insulin four years after the diagnosis (patient number 2.2).

Patients with mutations in other genes

One patient with a novel heterozygous *de novo* mutation in the *INS* gene (p.Cys95Trp) was diagnosed at the age of 4 months. He remains insulin-treated (patient number 4.4). One patient diagnosed at 15 months of age developed elevated levels of AST and ALT after one year, anemia, and leukopenia later during follow-up (patient number 11.14). He also had congenital stenosis of the aorta and skeletal dysplasia, which became evident after infancy. Anti-GAD was negative, and Wolcott Rallison syndrome was confirmed by the detection of two homozygous *EIF2AK3* mutation. He is still on insulin and supportive therapy (for orthopedic complications and autoimmune hepatitis) at the age of 14.5 years. Similarly, another unrelated patient diagnosed at 15 months of age showed elevated transaminase levels, persistent hyperkalemia, thrombocytopenia, and skeletal dysplasia after two years and was also found to be homozygous for the *EIF2AK3* mutation (patient number 12.15). His sister, diagnosed with NDM at four months of age, was homozygous for the same mutation (the patient number 12.16). Both patients are still on insulin and supportive treatment (for orthopedic and renal complications) at the age of 15.5 and 4.5 years.

Discussion

Although NDM is defined as diabetes diagnosed during the first six months of life, recent research shows that, rarely, it may present as late as 12 or even 24 months of age (1-4) although between the ages of 6 and 24 months a diagnosis of type 1 diabetes is much more common. The median age of diagnosis in our study was five months (4 days-18 months). The most striking finding in this investigation was the presentation of diabetes in a patient with a genetically confirmed NDM at 18 months of age. This patient and his two cousins were found to have a homozygous pathogenic *ABCC8* mutation, and after many years on insulin treatment, they were successfully switched to SU therapy. NDM genes must be therefore be considered when carefully collected family history suggests a possible genetic cause.

There was no statistical difference in terms of gender in our patients. Iafusco et al. (20) similarly reported no gender difference in their cohort.

In a study reported by Russo et al. (21), 75% of the patients with NDM diagnosed during the first six months of life had a mutation in *KCJN11*, *ABCC8*, or *INS* gene. This ratio dropped to 12% in patients diagnosed between 7-12 months. Russo et al. (21) also reported that the patients diagnosed with permanent NDM before six months of age without the mentioned mutations had higher birth weight than those with the mutations. In our smaller cohort, we did not observe a similar difference between patients with and without a causative mutation. By the findings of Besser et al. (22), more than 50% of our patients with NDM had low birth weight despite term delivery due to in utero hypoinsulinemia. Letourneau et al. (23) reported that 66.2% of the patients with monogenic diabetes presented with DKA similar to our patients, with 60% having DKA at the time of diagnosis.

Previous reports have suggested that autoantibodies are usually negative in NDM patients diagnosed before six months of age except for maternal autoantibodies, which may have crossed the placenta (24) and patients with monogenic autoimmunity such as IPEX syndrome (25). GAD-65 were positive, but anti-insulin antibodies were negative, in two of our patients diagnosed at seven months and four days (the patients' number 7.9 and 9.11). These patients did not have mutations in the known NDM genes (including monogenic autoimmunity genes such as *FOXP3, IL2RA,* and *LRBA)*, however more causal genes remain to be discovered and a monogenic etiology is therefore possible. Whilst the antibody positivity in the patient diagnosed at 7 months suggests a diagnosis of type 1 diabetes is likely, further investigations are needed to define the genetic etiology of the patient diagnosed at 4 days since antibody positivity is common in patients with neonatal diabetes caused by monogenic autoimmunity (26).

Molecular diagnosis in NDM is important for planning treatment and predicting prognosis. Therefore, genetic testing is essential in these patients. Carmody et al. have discussed the pros and cons of trying SU treatment awaiting the results of genetic tests. The advantages are a neurologic improvement, shorter hospital stay, lower cost, easier than insulin injections, and safety. On the other hand, increased expectance and disappointment of the family in case of treatment failure, risk of hypoglycemia in transient NDM, unknown long-term risks, and lack of FDA approval for SU in infants are the disadvantages (13). One of our patients was started on SU and responded well before the genetic test result was obtained, and insulin was successfully discontinued (patient number 5.5). After receiving the test results, all of the patients were switched to SU, and better glycemia was achieved along with significant elevation in C-peptide. Other family members with diabetes were also tested and switched to SU, which markedly improved their quality of life. Bowman et al. (15) had published a cohort of 90 patients with *KCNJ11* mutations causing permanent NDM followed for ten years. SU response was excellent in 93%, and neurologic development was improved by 47%. Similarly, we observed an excellent response to SU in 7 of 8 patients (87.5%) with mutations affecting the pancreatic potassium channel. Only one patient required the addition of long-acting insulin to the treatment.

Despite the importance of genetic diagnosis, it may not be possible in all patients as some etiological genes still remain to be discovered. De Franco et al. (16) has reported that a genetic mutation was detected in 82% of patients in an international cohort of 1200 probands. Similarly, we found a causative mutation in 87% of our patients.

The most common syndromic form of NDM in countries with high consanguinity rate is Wolcott Rallison syndrome (16). We had three patients with this syndrome, including two siblings born to consanguineous parents. All three patients had hepatic dysfunction and skeletal dysplasia, which are known features of the syndrome (27). They are on insulin treatment, and their diabetes is well controlled. Demirbilek et al. (28) investigated the genetic profile of the patients with NDM in Southeastern Turkey and found that mutations in potassium channel were less common in consanguineous families, and syndromic diabetes was more. In our findings, potassium channel mutations were more common in our patients, similarly to what is reported in Western countries.

Conclusions

The recognition of NDM increases with the identification of new genetic causes and the availability of genetic testing. Early diagnosis is essential to identify the patients who may respond to SU treatment. NDM is defined as diabetes diagnosed during the first six months of life, the presentation of NDM may be delayed. In rare cases, it may present as late as 12 or even 24 months of age therefore family history must be taken carefully. Most patients present before six months of age, and rapid genetic diagnosis must be obtained to plan the treatment. Syndromic diabetes must be considered in those with additional findings.

Limitations

There were some difficulties in reaching all of the data because of the retrospective nature of the study. The age range of the patients was wide, and some patients had antibody positivity, which rendered patient selection for the study difficult. The relatively small number of patients in this cohort is limited, and further research with a large number must be done.

Funding

Genetic testing at the Exeter genetic laboratory was funded by a Wellcome Trust senior investigator grant to Sian Ellard and Andrew Hattersley. EDF is a Diabetes UK RD Lawrence fellow.

Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of Bursa Uludag University(approval number: 2020-8/23).

Informed Consent: Consent form was filled out by all parents and participants.

Authorship Contributions

Surgical and Medical Practices: Elif Sobu, Ozgecan Demirbas, Yasemin Denkboy Ongen

Concept: Yasemin Denkboy Ongen, Erdal Eren, Omer Tarim

Design: Elif Sobu, Ozgecan Demirbas

Data Collection or Processing: Elisa De Franco, Elif Sobu

Analysis or Interpretation: Yasemin Denkboy Ongen, Erdal Eren, Sian Ellard

Literature Search: Omer Tarim, Sian Ellard, Elisa De Franco

Writing: Yasemin Denkboy Ongen, Erdal Eren, Elisa De Franco, Omer Tarim

Conflict of Interest: No conflict of interest

Financial Disclosure: No financial disclosure

References

1. Flechtner I, Vaxillaire M, Cavé H, et al. Neonatal hyperglycemia and abnormal development of the pancreas. Best Pract Res Clin Endocrinol Metab 2008;22(1):17-40.
2. Polak M, Cavé H. Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis 2007;2:12.
3. Aguilar-Bryan L, Byran J. Neonatal diabetes mellitus. Endocr Rev 2008;29:265-91.
4. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta cell diabetes. Nat Clin Pract End Met 2008;4:200-13.
5. Iafusco D, Massa O, Pasquino B, et al. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. Acta Diabetol 2012;49:405-8.
6. Habeb AM, Al-Magamsi MSF, Eid IM, et al. Incidence, genetics and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. Pediatr Diabetes 2012;13:499-505.
7. Flanagan SE, De Franco E, Lango Allen H, et.al. Analysis of transcription factors key fos mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. Cell Metab 2014;19:146.
8. Rubio-Cabezas O, Ellard S. Diabetes mellitus in neonates and infants: genetic heterogeneity, clinical approach to diagnosis, and therapeutic options. Horm Res Paediatr 2013;80(3):137-46.
9. Gloyn AL, Pearson ER, Antcliff JF, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2and permanent neonatal diabetes. N Engl J Med 2004;350(18):1838-49.
10. Pearson ER, Flechtner I, Njølstad PR, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006;355(5):467-7.
11. Tonini G, Bizzarri C, Bonfanti R, et al. Sulfonylurea treatment outweighs insulin therapy in short-term metabolic control of patients with permanent neonatal diabetes mellitus due to activating mutations of the KCNJ11 (KIR6.2) gene. Diabetologia. 2006;49(9):2210-3.
12. Zhang H, Zhong X, Huang Z, et al. Sulfonylurea for the treatment of neonatal diabetes owing to KATP-channel mutations: a systematic review and meta-analysis. Oncotarget. 2017;8(64):108274-85.
13. Carmody D, Bell CD, Hwang JL, et al. Sulfonylurea treatment before genetic testing in neonatal diabetes: pros and cons. J Clin Endocrinol Metab. 2014;99(12):2709-14.
14. Babiker T, Vedovato N, Patel K, et al. Successful transfer to sulphonylureas in KCNJ11 neonatal diabetes is determined by the mutation and duration of diabetes. Diabetologia 2016;59:1162.
15. Bowman P, Sulen A, Barbetti F, et al. Effectiveness and safety of long-term treatment with sulfonylureas in patients with neonatal diabetes due to KCNJ11 mutations: an international cohort study. Lancet Diabetes Endocrinol. 2018;6(8):637-46.
16. De Franco E, Flanagan SE, Houghton JA. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes:an international cohort study. The Lancet 2015; 386:957-63.
17. Ellard S. Baple EL, Berry I. et al. 2019. ACGS Best Practice Guidelines for Variant Classification 2019. Retrieved from https://www.acgs.uk.com/media/11285/uk-practice-guidelines-for-variant-classification-2019-v1-0-3.pdf
18. Proks P, Girard C, Haider S, et al. A gating mutation at the internal mouth of the Kir6.2 pore is associated with DEND syndrome. EMBO 2005;6:470-5.
19. Proks P1, Antcliff JF, Lippiat J et al. Molecular basis of Kir6.2 mutations associated with neonatal diabetes or neonatal diabetes plus neurological features. Proc Natl Acad Sci U S A. 2004;101(50):17539-44.
20. Iafusco D, Stazi MA, Cotichini R. Permanent diabetes mellitus in the first year of life. Diabetologia 2002;45:798-804.
21. Russo L, Iafusco D, Brescianini S. Permanent diabetes during the first year of life: multiple gene screening in 54 patients. Diabetologia 2011;54:1693-701.
22. Besser REJ, Flanagan SE, Mackay DGJ. Prematurity and genetic testing for neonatal diabetes. Pediatrics. 2016;138(3).
23. Letourneau L, Carmody D, Wroblewski K, et al. Diabetes Presentation in Infancy: High Risk of Diabetic Ketoacidosis. Diabetes Care 2017;40(10):147-8.
24. Ziegler AG, Hummel M, Schenker M et al. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. Diabetes. 1999;48(3):460-8.
25. Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr. 1982;100(5):731-7.
26. Johnson MB, Hattersley AT, Flanagan SE. Monogenic autoimmune diseases of the endocrine system. *Lancet Diabetes Endocrinol*. 2016;4(10):862-872. doi:10.1016/S2213-8587(16)30095-X.
27. Delepine M, Nicolino M, Barrett T et al. EIF2AK3, encording translation ignition factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome, Nat Genetic 2000;25:406.
28. Demirbilek H, Arya VB, Ozbek MN et al. Clinical characteristics and molecular genetic analysis of 22 patients with neonatal diabetes from the South-Eastern region of Turkey: predominance of non-KATP channel mutations. European Journal of Endocrinology. 2015;172(6):697-705.

Table 1. The age of diagnosis, genetic analysis, and treatment response of NDM patients

Family and patient number	**Age (d/m/y)**	**Current age (year)**	**Sex**	**Consanguinity**	**HbA1c at diagnosis**	**C-peptide at diagnosis**	**Gene**	**Location**	**DNA-protein description**	**Variant classification according to ACMD guidelines**	**Consequence**	**Zygosity**	**NDM subtype**	**Treatment**	**SU response**
1.1	3 m	6.5	F	No	11.8	0.01	*KCNJ11*	Exon 1	c.175G>A p.Val59Met (p.V59M)	Pathogenic	Missense	Heterozygous	Permanent	SU	Yes
2.2	2.5 m	6	F	No	N/A	N/A	*KCJN11*	Exon 1	c.175G>A p.Val59Met (p.V59M)	Pathogenic	Missense	Heterozygous	Permanent	SU+insulin	Yes (has been added insulin treatment four years later)
3.3	40 d	6	F	No	10.5	0.07	*KCJN11*	Exon 1	c.175G>A p.Val59Met (p.V59M)	Pathogenic	Missense	Heterozygous	Permanent	SU	Yes
4.4	4 m	5	M	No	9.9	0.75	*INS*	Exon 3	c.285C>G p.Cys95Trp (p.C95W) **	Likely Pathogenic	Missense	Heterozygous	Permanent	İnsulin	-
5.5	45 d	9	M	No	N/A	N/A	Unknown	-	-	-	-	-	Permanent	SU	Yes
6.6	18 m	23	F	Yes (1st degree cousins)	10.8	1.22	*ABCC8*	Exon 7	c.1144G>A p.Glu382Lys (p.E382K)	Pathogenic	Missense	Homozygous	Permanent	SU	Yes
6.7	9 m	27	M	Yes (1st degree cousins)	N/A	N/A	*ABCC8*	Exon 7	c.1144G>A p.Glu382Lys (p.E382K)	Pathogenic	Missense	Homozygous	Permanent	SU	Yes
6.8	18 m	36	M	Yes (1st degree cousins)	N/A	N/A	*ABCC8*	Exon 7	c.1144G>A p.Glu382Lys (p.E382K)	Pathogenic	Missense	Homozygous	Permanent	SU	Yes
7.9	7 m	5.5	F	Yes (1st degree cousins)	6.7	0.02	No disease-causing variant identified (anti GAD65	-	-	-	-	-	Permanent	İnsulin	-

							ab positive)								
8.10	12 d	8	F	No	6.5	0.06	ABCC8	Exon 28	c.3548G>A p.Arg1183Gln (p.R1183Q)	Likely Pathogenic	Missense	Heterozygous	Transient	İnsulin	-
9.11	4 d	Died	M	Yes (2nd degree cousins)	5.8	0.9	No disease- causing variant identified (anti GAD65 ab positive)	-	-	-	-	-	Permanent	İnsulin	-
10.12	6 m	1	F	Yes (1st degree cousins)	17.1	0.01	*ABCC8*	Exon 29	c.*692G*>T p.Trp231Leu**	Likely Pathogenic	Missense	Homozygous	Permanent	SU	Yes
10.13	8 m	3	F	Yes (1st degree cousins)	N/A	0.1	*ABCC8*	Exon 29	c.692G>T p.Trp231Leu**	Likely Pathogenic	Missense	Homozygous	Permanent	SU	Yes
11.14	15 m	14.5	M	No	13.1	N/A	*EIF2AK3*	Exon 13	p.Glu926Lys** p.Lys939Arg**	Likely Pathogenic	Missense	Homozygous	Permanent	İnsulin	-
12.15	17 m	15.5	M	Yes (2nd degree cousins)	N/A	N/A	*EIF2AK3*	Exons 11-13	c.1886_(c.2817+1_c.2818- 1) del p.?	Pathogenic	Partial gene deletion	Homozygous	Permanent	İnsulin	-
12.16	3.5 m	4.5	F	Yes (2nd degree cousins)	9.7	0.48	*EIF2AK3*	Exons 11-13	c.1886_(c.2817+1_c.2818- 1) del p.?	Pathogenic	Partial gene deletion	Homozygous	Permanent	İnsulin	-

N/A: not applicable
SU: **sulphonylurea**
** Novel mutations

Table 2. Before and after SU treatment values of C-peptide and HbA1c levels of NDM patients with ATP-dependent potassium channel mutations

Family and patient number	HbA1c at diagnosis and after SU treatment		C-peptide at diagnosis and after SU treatment	
1.1	11.8	5.9	0.01	2.31
2.2	NA	6.8	NA	2.89
3.3	10.5	6.3	0.07	1.11
5.5	NA	7.1	NA	1.12
6.6	10.8	9.3	1.22	5.8
6.7	NA	8.1	NA	3.18
6.8	NA	7.4	NA	2.5
10.12	17.1	9.6	0.01	3
10.13	NA	8.1	0.1	3

N/A: not applicable
SU: sulphonylurea